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Development of metal-ion containing catalysts for the decomposition of phosphorothioate esters ☆

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ABSTRACT

The widespread use of phosphorothioate esters as agricultural pesticides, chemical weapons and mechanistic probes in enzymology has sparked interest in the reactivity of these thio-substituted analogues of phosphate esters. In this brief account, we summarize the recent developments in our understanding of the mechanisms of hydrolysis (and solvolysis in methanol) of phosphorothioates containing a sulfur atom in the bridging and/or non-bridging position. A small number of highly efficient catalytic systems containing the metal ions La(III), Pd(II), Cu(II) and Zn(II) have been developed to promote the degradation of the various classes of phosphorothioate esters. The mechanisms of the base promoted solvolytic reactions in water and methanol and those of the metal catalyzed cleavage are presented, as well as a discussion of the energetics of the catalytic processes and other salient features. The aim of this review is to provide the reader with a contemporary physical organic description of phosphorothioate ester cleavage. This article is part of a Special Issue entitled: Chemistry and mechanism of phosphatases, diesterases and triesterases.

1. Introduction

Phosphorothioate esters such as the aryl-containing species **1** (Chart 1) comprise analogues of phosphate esters in which one or more of the oxygen atoms in a non-bridging position have been substituted with sulfur. Several of the neutral members of this class such as *O*,*O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate (**2**) and parathion (*O*,*O*-diethyl *O*-4-nitrophenyl phosphorothioate (**3**) are important in agriculture where they have seen widespread use as pesticides [1]. Species containing one or more sulfurs in the bridging positions, such as VX (**4**) and Russian VX (**5**), are also termed phosphorothioates. These compounds are powerful acetylcholine esterase inhibitors and have seen use as organophosphorus nerve agents [2].

Phosphorothioate diesters (**1b**) and monoesters (**1c**) have been employed as mechanistic probes for enzyme catalyzed phosphoryl transfer reactions to deduce transition state structure [3] and sites of metal ion coordination [4]. Phosphorothioate modification of synthetic oligonucleotides provides an increased stability toward nucleases [5] giving useful applications in molecular biology [6]. Examples of naturally occurring phosphorothioate DNA linkages have been identified [7].

Several reviews of the reactions of phosphates exist [8], but less attention has been directed toward the related chemistry of phosphorothioates; the state of knowledge up to 2005 with particular reference to physical organic chemistry has been summarized [9,10]. This brief account summarizes some of the more recent results concerning the phosphorothioyl group transfer between oxyanions as in the hydrolysis and transesterification reactions. In addition, we will summarize the most recent work on solvolytic alcoholysis reactions, particularly those reporting mechanistic studies of small molecules which are promoted by metal ions in solution.

2. Features of the reactions of phosphorothioates with S in non-bridging position

A pictorial representation of the bond-forming, bond-breaking processes occurring in various concerted phosphoryl/phosphorothioyl transfer reactions that phosphate and phosphorothioate mono-, di- and triesters (**1a–c**) undergo [10] is presented in the More-O'Ferrall-Jencks diagram in Fig. 1. Purcell and Hengge have reported the activation parameters for the hydrolysis of *p*-nitrophenyl substituted phosphate and phosphorothioate pairs (**1a**,**b**,**c**; X = O,S) which are reproduced in Table 1 [9a]. The general feature of note is that the hydrolysis of each phosphorothioate has a higher $\Delta H^{\ddagger}$ than its corresponding phosphate by 4–6 kcal/mol, and has more favorable (more positive) $\Delta S^{\ddagger}$. The net effect on the kinetics of reaction is termed the 'thio effect' which is

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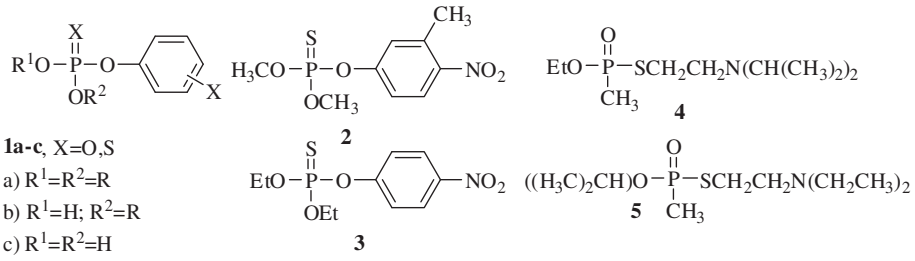


Chart 1. Chemical structures of various neutral phosphorothioates.

represented as k^O/k^S which, at 298 K, is 12.6 for the hydrolysis of **1a**,X = O/**1a**,X = S, 3.3 for hydrolysis of **1b**,X = O/**1b**,X = S, and 0.13 for the hydrolysis of the dianions of **1c**,X = O/**1c**,X = S. These are small numbers in the case of the solvent mediated reactions and, given the differences in the $\Delta H^{\ddagger}$ for phosphates and their corresponding phosphorothioates, are expected to vary with temperature, possibly becoming inverted depending on which side of the isokinetic temperature the reactions are studied.

2.1. Solvolysis of diesters and monoesters (**1b**/**1c**)

The hydrolysis of phosphorothioate monoester dianions **1c**,X = S,$R_1 = R_2 = {}^{(-)}$ with aryloxy leaving groups is thought to proceed by a highly dissociative ($D_N + A_N$) mechanism involving formation of a metaphosphorothioate intermediate (O_2PS^-, bottom right corner of Fig. 1). This proposal is consistent with: 1) the large negative β^{Lg} of −1.1 determined for the hydrolysis of aryl phosphorothioate dianions; 2) the large positive entropy of activation ($\Delta S^{\ddagger}$ of + 29 cal/mol/K) for reaction of 4-nitrophenyl phosphorothioate dianion; and 3) the racemization observed in the hydrolysis of chiral 4-nitrophenyl [^{16}O,^{18}O]phosphorothioate [9a,11,12]. Heavy atom kinetic isotope effects indicate that the leaving group has undergone significant P–OAr cleavage and bears nearly a full negative charge at the transition state for hydrolysis of 4-nitrophenyl phosphorothioate dianion [13]. The hydrolytic mechanisms of phosphorothioate diesters (**1b**) have not been as extensively investigated to date. Nonetheless, kinetic isotope effects indicate that the transition state for hydrolysis of the diester, *O*-ethyl *O*-4-nitrophenyl phosphorothioate (**1b**, X = S, R_1 = Et, $R_2 = {}^{(-)}$), involves less P–OAr bond cleavage relative to the monoester but more than the triester [13].

2.2. Solvolysis of O,O-dialkyl O-aryl phosphorothioates (**1a**)

The three main modes of nucleophilic cleavage for phosphorothioate triesters (**1a**,X = S) are illustrated in Scheme 1 for the decomposition of fenitrothion (**2**) in basic ethanol. Nucleophilic attack of ethoxide on the aryl group of **2** leading to C–O bond cleavage competes with attack on the phosphorus and fission of the P–OAr bond. The rate constants for these two processes are similar in ethanol ($k_2 \approx 6\times10^{-5}\ M^{-1}\ s^{-1}$ at 25 °C) affording near equal amounts of the C–O and P–O bond cleavage products [14]. Selectivity for the P–O bond cleavage products is higher in methanol [15] at 93:7 and essentially quantitative in water [16]. A minor amount of product, (~5%) from nucleophilic aromatic substitution, is obtained in ethanol but this process does not generally compete significantly with the other two modes of cleavage.

Table 1
Activation parameters for the aqueous cleavage of the *p*-nitrophenyl derivatives of phosphate and phosphorothioate esters **1a**,**b**,**c**; X = O,S at 312 K under basic conditions.[a]

Substrate	$\Delta G^{\ddagger}$ (kcal/mol)	$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta S^{\ddagger}$ (cal/mol/K)
Monoester dianion			
1c, $R_1 = R_2 = {}^{-}$, X = O	29.5	30.6	+3.5
1c, $R_1 = R_2 = {}^{-}$, X = S	27.9 ± 1.0	37.0 ± 1.0	+29 ± 3
Diester, monoanion			
1b, R_1 = Et; $R_2 = {}^{-}$, X = O	27.4 ± 0.5	14.91 ± 0.06	−36.3 ± 0.1
1b, R_1 = Et; $R_2 = {}^{-}$, X = S	27.9 ± 0.4	18.5 ± 0.1	−26.9 ± 0.3
Triester			
1a, $R_1 = R_2$ = Et, X = O	20.0 ± 0.3	12.69 ± 0.03	−24.81 ± 0.05
1a, $R_1 = R_2$ = Et, X = S	21.5 ± 0.2	16.43 ± 0.08	−17.3 ± 0.1

[a] Data reproduced from ref. [9a].

2.3. Mechanistic details of P–OAr fission from triesters

Linear free energy relationships for the transfer of the *O*,*O*-dimethyl phosphorothioyl (S=P(OCH$_3$)$_2$) group between aryloxy anions in water are consistent with a concerted process involving simultaneous bond formation with the incoming nucleophile and bond rupture of the nucleofuge [17,18]. A charge map for the symmetrical reaction between the weak nucleophile, 2,4,5-trichlorophenoxide, and *O*,*O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate in water is shown in Scheme 2 [18]. On the basis of the experimental evidence, the activated complex is described as slightly loose, where bond cleavage has progressed to the extent of 61% defined in terms of the Leffler parameter, α, which relates the extent of bond cleavage or formation by comparing the Brønsted β^{Lg} for the TS to the β^{Eq} for equilibrium

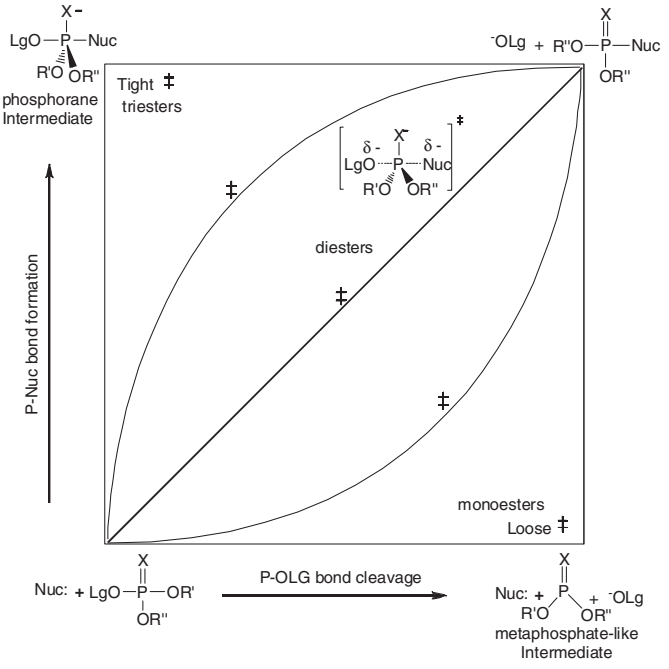
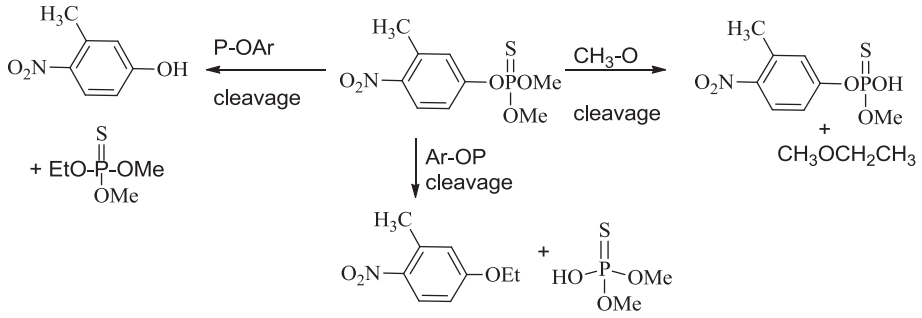


Fig. 1. A More-O'Ferrall-Jencks diagram showing bond forming and bond cleaving axes, and the types of transition structures for nucleophilic substitution of the mono-, di-, and triesters of phosphates (X = O) and phosphorothioates (X = S).



Scheme 1. Nucleophilic reactions of fenitrothion in basic ethanol.

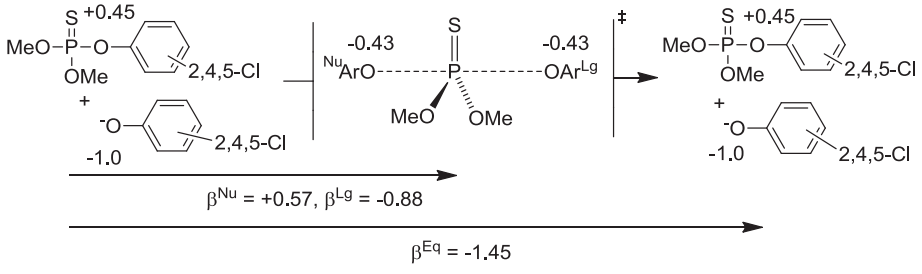
transfers of acyl or phosphoryl groups between oxyanion nucleophiles. By this measure, $\alpha = \beta^{Lg}/\beta^{Eq} = -0.88/-1.45 = 0.61$ whereas bond formation has only progressed to an extent of 39% ($\alpha = \beta^{Nu}/\beta^{Eq} = |0.57/-1.45| = 0.39$). A tighter, more associative, transition state with less cleavage to the leaving group is indicated for the attack of the stronger nucleophile hydroxide on *O,O*-dimethyl *O*-aryl phosphorothioates, where $\alpha = \beta^{Lg}/\beta^{Eq} = 0.34$ [15]. For attack of hydroxide on *O,O*-dimethyl *O*-4-nitrophenyl phosphorothioate, heavy atom kinetic isotope effects support a reduced degree of P–OAr bond cleavage in the transition state relative to the reaction of the phosphate triester analogue [13]. The *O*-4-nitrophenyl dimethylphosphinothioate ester undergoes alkaline hydrolysis some 2000 times faster than *O,O*-dimethyl *O*-4-nitrophenyl phosphorothioate [19] via a process described as concerted based on a combination of linear free energy relationship ($\beta^{Lg} = -0.54$, $\beta^{Nu} = 0.47$ for aryloxy nucleophiles) and heavy atom kinetic isotope effect data. Corroborating evidence comes from the $^{18}O/^{16}O$ kinetic isotope effect for the bridging *p*-nitrophenoxy of 1.012 which indicates that the P–O bond to the leaving group is ~40% broken at the transition state. A considerable amount of data concerning the cleavage of phosphorothioates has been accumulated in methanol solvent. This necessitates the measurement of pH and pK_a values for various species such as phenols, HOAr, in methanol and the latter values are, respectively, designated as ${}^{s}_{s}pH$ and ${}^{s}_{s}pK_a^{HOAr}$ indicating that pH or pK_a is measured in, and referenced to, that solvent [20]. Shown in Fig. 2 are experimentally determined Brønsted plots (of $\log(k_2^{-OMe})$ vs. the ${}^{s}_{s}pK_a^{HOAr}$ for the dissociation of the conjugate acid of the phenoxide leaving group) for the methoxide-promoted cleavage of **1a**,X = S,$R_1 = R_2 = CH_3$, (■) and **1a**,X = O,$R_1 = R_2 = CH_3$, (◇) reacting by P–OAr bond fission in anhydrous methanol [15]. The gradients of the plots for both substrates with aryloxy leaving groups are similar providing $\beta^{Lg} = -0.50$ ($\alpha = 0.36$) and -0.59 ($\alpha = 0.32$), respectively [15,21]. The 10- to 100-fold greater reactivity of phosphate triesters relative to their analogous phosphorothioates with the same leaving groups is clearly demonstrated by the vertical separation of the best fit lines in Fig. 2.

DFT calculation of the free energies for the methanolysis of **1a**,X = S, $R_1 = R_2 = CH_3$ reveals that nucleophilic attack on all aryloxy containing substrates occurs along a trajectory opposite to the leaving group (see Scheme 3) [15]. Nevertheless, although this step is rate limiting, there is a gradual change from a one step to two step process as the leaving group becomes poorer. Substrates **1a**,X = S,$R_1 = R_2 = CH_3$ with activated leaving groups (${}^{s}_{s}pK_a^{HOAr} < 12.3$) were shown to react by an enforced concerted (A_ND_N) [term defined in ref. 22] mechanism whereby TS_{Nu} involves considerable shortening of the nucleophile-P distance with very little lengthening of the P–OAr bond as indicated by the More O'Farrell-Jencks diagram in Fig. 3(a). Subsequently, this TS_{Nu} proceeds to products by a barrierless lengthening of the P–OAr bond. Methanolysis of the less activated substrates having ${}^{s}_{s}pK_a^{HOAr} > 12.3$ proceeds through ***TS*$_{Nu}$** to a stable 5-coordinate thiophosphorane intermediate (Int) in a stepwise ($A_N + D_N$) mechanism followed by fast breakdown of ***INT*** via ***TS*$_{Lg}$** (see More O'Farrell-Jencks diagram in Fig. 3(b)). Similar computations also show that a transition from concerted to stepwise mechanisms as a function of leaving group ability is also operational for the base promoted solvolysis of phosphate triesters in methanol and water [15,23].

3. Solvolysis of phosphorothioates with S in bridging position

3.1. S-aryl phosphorothioates, phosphonothioates, and phosphorothiolates

Interest in the solvolyses of S-aryl containing neutral phosphorothioates, phosphonothioates (**6**,**7**) and anionic phosphonothioates (**8**) stems partly from their use as simulants for the study of the degradation of chemical weapons such as VX (**4**) and Russian-VX (**5**). Phosphorothioates have also been reported to be versatile synthetic reagents undergoing both α and γ selective allylic alkylation in addition to other reactions [24]. Alkaline methanolysis of *O,O*-diethyl *S*-aryl phosphorothioates (**6**) is some 10 to 30 times faster than the corresponding *O,O*-diethyl *O*-aryl phosphates (**1a**,X = O,$R_1 = R_2 = Et$) at 25 °C [25]. The corresponding Brønsted plots for the phosphorothioates and phosphates are similar at $\log k_2^{-OMe} = (6.00 \pm 0.86) - (0.76 \pm 0.08)\ {}^{s}_{s}pK_a^{HSAr}$ and $\log k_2^{-OMe} = (6.20 \pm 0.70) - (0.70 \pm 0.05)\ {}^{s}_{s}pK_a^{HOAr}$ respectively, revealing that the increased rate of reaction for esters **6** over the range investigated is largely attributable to the lower ${}^{s}_{s}pK_a$ of the leaving group thiols relative to phenols. However, if the change in ionization constant accompanying substitution of oxygen for sulfur is accounted for by plotting $\log k_2^{-OMe}$ vs. leaving group ${}^{s}_{s}pK_a$, the phosphate triesters are revealed to be ~5 fold *more* reactive. The rates of nucleophilic attack on phosphonates are



Scheme 2. Charge map for the symmetrical reaction between 2,4,5-trichlorophenoxide and *O,O*-dimethyl *O*-2,4,5-trichlorophenyl phosphorothioate in water.

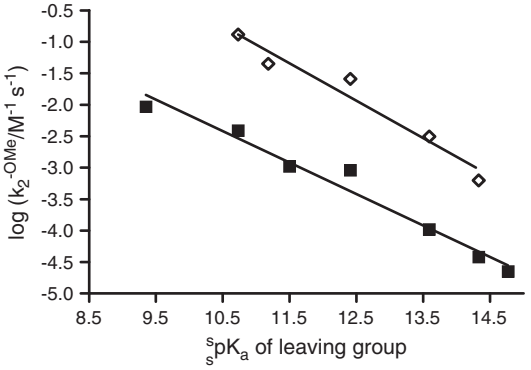
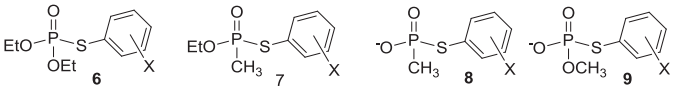


Fig. 2. Plots of log (k_2^{-OMe}) vs. the $^{s}_{s}pK_a$ of the leaving group phenols for the methoxide promoted cleavages of *O*,*O*-dimethyl *O*-aryl phosphorothioates (**1a**,X = S,R_1 = R_2 = CH_3) and *O*,*O*-dimethyl *O*-aryl phosphates (**1a**,X = O,R_1 = R_2 = CH_3) reacting by P–OAr bond cleavage in anhydrous methanol. (Redrawn from ref. [15]).

typically greater than on the corresponding phosphates and expectedly the *O*-ethyl *S*-aryl methylphosphonothioate esters (**7**) are up to 30-fold more reactive toward methoxide-promoted cleavage than **6** [26]. The corresponding Brønsted plot reveals a moderate rate dependence on the $^{s}_{s}pK_a$ of the arylthiolate leaving groups ($\beta^{Lg} = -0.65$) that can be used to estimate a half-time of 650 years for the methanolytic cleavage of the neurotoxin VX at a near neutral $^{s}_{s}$pH of 9 and 25 °C [26].

Degradation of VX and Russian-VX by alkaline hydrolysis can be problematic owing to their propensity to undergo a P–OR cleavage in competition with the desired P–SR cleavage reaction. For example, hydrolysis of VX in a 0.1 M NaOH solution as in Eq. (1) produces 87% of the desired cleavage product, *O*-ethyl methylphosphonate (EMPA), and 13% of an undesirable de-ethylated product, *S*-(N,N-(diethylaminoethyl) methylphosphonate) commonly known as EA2192 [27]. A study of the methoxide promoted cleavage of EA2192 simulants **8** demonstrated that these anionic *S*-aryl methylphosphonothioate esters are more resistant to degradation than the neutral esters **3** by factors exceeding 10^5 in certain cases [28]. The Brønsted coefficient ($\beta^{Lg} = -0.85$) for the methoxide-promoted methanolysis of series **8** leads to a prediction that EA2192 has a half-life of ~10^6 years in neutral $^{s}_{s}p^{s}H$ methanol at 25 °C. EA2192, formed as a by-product of VX hydrolysis, is highly resistant toward further solvolytic degradation and yet retains a high level of oral and i.v. toxicity. For this reason, alternate strategies for the degradation of VX that circumvent EA2192 production are much sought after [29], including approaches using biological glycerophosphodiesterases [30] and metallocene complexes [31].

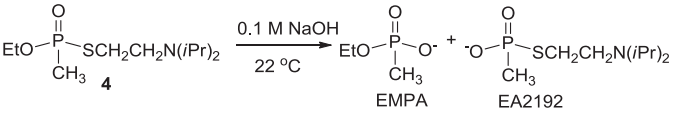


Anionic *O*-methyl *S*-aryl phosphorothiolates (**9**) react with substituted pyridines by a nucleophilic mechanism producing pyridine methyl phosphoroamidates [32]. The Leffler parameters for attack of 3-picoline on diesters **9** and *O*-methyl *O*-aryl phosphates (**1b**,X = O,R_1 = CH_3, R_2 = $^{(-)}$) are very similar at $\alpha = \beta^{S}_{Lg}/\beta^{S}_{Eq} = 0.79$ and $\beta^{O}_{Lg}/\beta^{O}_{Eq} = 0.75$ for the SP and OP diesters, respectively. The β^{Nu} for attack of substituted pyridines on these two substrate classes are 0.42 and 0.56 leading to the conclusion that Piccirilli [32] that nucleophiles react with SP and OP diesters by similar mechanisms and transition structures. A complementary conclusion was reached based on the kinetic isotope effects for the leaving group and nucleophile in the intramolecular transesterification of *O*-2-hydroxypropyl *O*-4-nitrophenyl phosphate and *O*-2-hydroxypropyl *S*-3-nitrobenzyl phosphorothiolate in water [33,34].

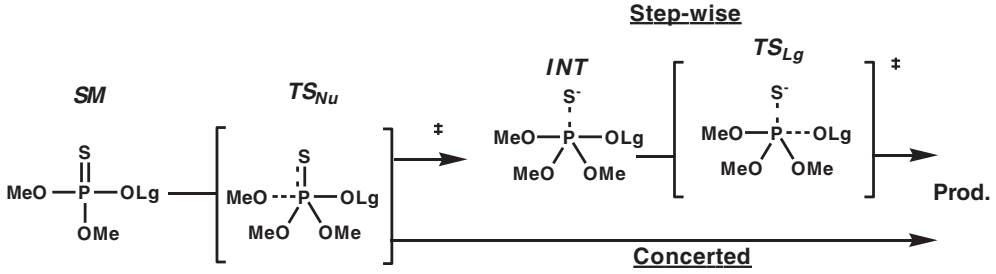
4. Metal ion catalyzed solvolysis of phosphorothioates

4.1. Studies of the hydrolysis and methanolysis of derivatives with S in a bridging position (P–SAr and P–SR)

A preliminary study of the methanolysis reaction of **6** (X = H, *p*-NO_2) promoted by 1 mM La(III) and 1 mM Cu(II):(1,5,9-triazacyclododecane), (**10**:Cu), each containing equimolar $^{-}$OMe, showed that the methanolytic displacement of $^{-}SC_6H_4NO_2$ from **6** was accelerated by 5.3×10^6 ($^{s}_{s}$pH = 9.03 for La(III)) and 8.3×10^6 for the Cu(II) complex ($^{s}_{s}$pH = 8.75) [35]. Studies showed that the 1:1 metal-methoxide forms, $M^{x+}(^{-}OCH_3)$, were the most active ones leading to a proposed mechanism that involves transient pre-equilibrium binding of **6** to **10**:Cu(II):($^{-}OCH_3$), followed by an intramolecular delivery of methoxide with concerted displacement of the S-aryl group as in **11**. The most active species in the La(III) system are dimers such as $(La(III):(^{-}OCH_3))_2$ that have been suggested [25] to promote the cleavage of phosphate, phosphonate, phosphorothioate, and phosphonothioate esters by the generalized mechanism given in Scheme 4.



A more comprehensive study [25] of the La(III) and **10**:Zn(II):($^{-}OCH_3$) promoted methanolysis of a series of phosphate triesters **1a**,X = O,R_1 = R_2 = Et and S-aryl triesters **6** provided the linear free energy Brønsted β^{Lg} values listed in Table 2. Also included for comparison are values determined for the cleavage of **6**–**8**. The notable features of the plots are the large β^{Lg} values observed for the metal ion promoted methanolysis of the phosphate (**1a**,X = O,R_1 = R_2 = Et) and phosphonate (**7**, S = O) derivatives. Each of these indicates that



Scheme 3. Reaction map showing species considered in the calculations for lyoxide reactions of **1a**,X = S,R_1 = R_2 = CH_3. [15].

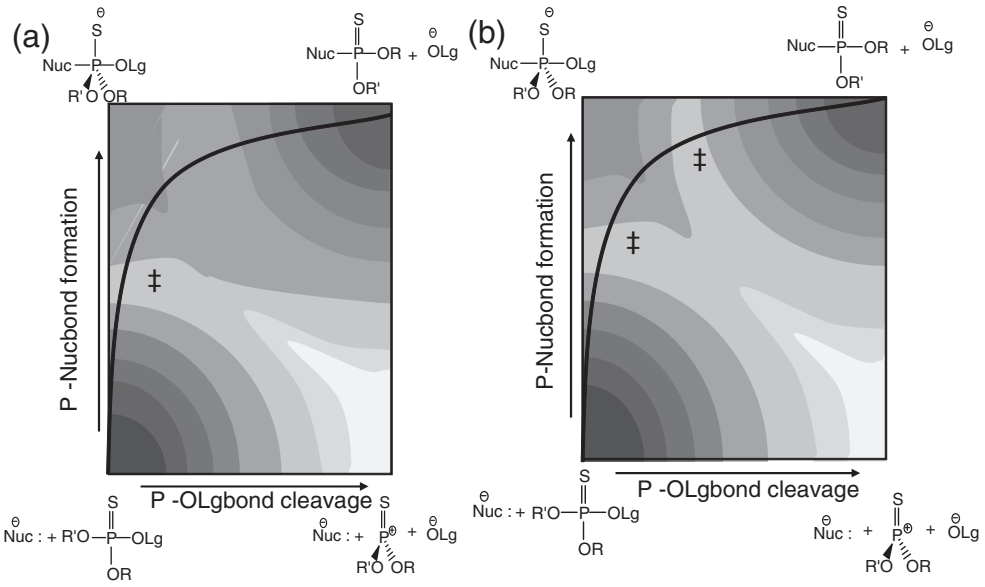
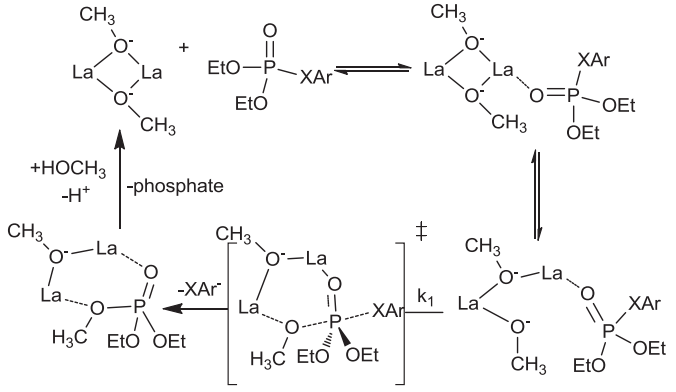


Fig. 3. (a) More O'Ferrall-Jencks (MO-J) diagrams for the enforced concerted pathway for attack of a good nucleophile (hydroxide or methoxide) on a phosphorothioate triester with a good leaving group (3,5-dichlorophenoxy or better). A single transition state with considerable formation of the Nuc–P bond and little change in the P–OAr bond length, followed by a barrierless expulsion of leaving group; (b) Diagram for two step reaction of good nucleophile on phosphorothioate with poorer leaving group. In this case the reaction is symmetrical with identical leaving group and nucleophile.

there is apparently a far greater degree of cleavage of the P–O-aryl leaving group bond in the rate limiting step of the reaction than is the case with the methoxide promoted reactions. If one assumes for series **1a**,X = O,R_1 = R_2 = Et, that the β^{Eq} value of −1.87 for equilibrium transfer of the $(Et)_2$-P = O unit between oxyanion nucleophiles in water [37] transcribes to methanol, then the $\alpha = \beta^{Lg}/\beta^{Eq} = 0.76$ for the La(III) reaction and 0.60 for the **9**:Zn(II):($^-OCH_3$) reaction. In the simplest analysis, each of these shows considerably more leaving group cleavage than does the methoxide reaction where $\alpha = 0.37$. The large amount of cleavage in the metal ion catalyzed reaction could be a result of a late transition state in a concerted reaction, or a movement to a two-step process, where the rate limiting step is breakdown of a 5-coordinate phosphorane intermediate. Although there is not an experimentally known β^{Eq} for transfer of the $(EtO)_2P$ = O group between oxyanions and thiolates, one might surmise from literature data that the substitution of O-aryl by S-aryl will reduce the β^{Eq} for the reaction of **6** to from −1.87 to −1.5 or −1.6 [38]. Thus the α value for cleavage of substrates **6** by La(III) and **10**:Zn(II):($^-OCH_3$) is ~0.6–0.5 and 0.5 respectively, meaning there is significantly less cleavage of the P–S-aryl bond in the transition state.

Linear free energy studies of certain of the methoxide and La(III)-promoted reactions of substrate series **7** and **8** allow one to be able to make predictions of the rate of catalytic decontamination of EA2192 and VX respectively. The β^{Lg} for the methoxide reaction of **8** is slightly larger than observed for the neutral substrates in Table 2, but the reactions are much slower due to repulsion of the anionic nucleophile and substrate. The second order rate constants range from 4.7×10^{-5} to 1.5×10^{-7} M^{-1} s^{-1} for a series of substrates with $^{s}_{s}pK_a^{HSAr}$ of ~9 to 11, log $(k_2^{-OMe}) = (-0.85\pm0.02)$ $^{s}_{s}pK_a^{HSAr} + (3.40\pm0.17)$. Due to the strong attraction of the positively charged catalyst and negatively-charged substrate, the La(III)-catalyzed reaction at $^{s}_{s}$pH 8.4 (close to neutrality) exhibits saturation kinetics indicative of equilibrium formation of a strongly bound complex $(La(III)_2$:**8**:$(^-OCH_3))_2$ which undergoes spontaneous decomposition to release the S–Ar group. The complex promoted cleavage of a given substrate **8** is accelerated by $(3–9)\times10^{10}$ relative to its methoxide promoted reaction at $^{s}_{s}$pH 8.4. Based on this acceleration, it is predicted that the $t_{1/2}$ for La(III)-catalyzed cleavage of EA2192 will be 4 min at $^{s}_{s}$pH 8.4 [28]. A similar analysis using substrates **7** indicated that VX would be destroyed by a 1 mM solution of $(La(III):(^-OCH_3))_2$ in methanol at 25 °C with a $t_{1/2}$ of 0.3 s [30].

Until recently, the cleavage of these sorts of acyclic phosphate and phosphorothioate triesters promoted by HO^- and CH_3O^- was believed to occur via a concerted mechanism for substrates having good leaving groups [39]. However, more recent computational results [15,23] indicate that these reactions are complex and have a spectrum of possibilities depending on leaving group ability. At one end of the spectrum, this involves a single rate-limiting attack of the



Scheme 4. Proposed mechanism of the $(La(III):(^-OCH_3))_2$ promoted cleavage of phosphate (**1a**, X = O) and phosphorothioate (**1a**, X = O) triesters. Redrawn from ref. [25]; metal ion charges omitted for clarity.

Table 2
Brønsted β^{Lg} values determined for the La(III) and **9**:Zn(II):($^-OCH_3$) promoted methanolysis of **1a**, X = O, R_1 = R_2 = Et, and **6**–**8**, T = 25 °C.

β^{Lg}, (catalyst) → substrate ↓	β^{Lg} ($^-OCH_3$)	β^{Lg} ($La^{(III)}$: $(^-OCH_3))_2$	**10**:Zn(II): ($^-OCH_3$)
1a,X = O,R_1 = R_2 = Et [25]	−0.70	−1.43	−1.12
6 [25]	−0.63	−0.87	−0.74
7 [26]	−0.65	−0.75	−0.66
7,(S = O) [36]	−0.76	−1.26	−1.06
8 [28]	−0.85	−0.67[a]	

[a] Conducted at $^{s}_{s}$pH 8.4, where 96% of the reaction proceeds by way of the decomposition of the catalyst:substrate complex, $(La^{2+})_2$:**8**:$(^-OCH_3)_2$.

like transition state followed by barrierless breakdown to product with substrates having good aryloxy leaving groups. In the latter cases, the reaction is said to be 'enforced concerted', meaning that the 5-coordinate species has a lifetime shorter than a bond vibration. However, as the leaving groups become poorer, the mechanism switches to two steps with a discrete intermediate. The 5-coordinate intermediate in this case becomes increasingly difficult to cleave to products as the leaving group becomes progressively poorer. Ultimately the expulsion of the leaving group is rate-limiting when leaving group departure is more difficult than the expulsion of the nucleophile.

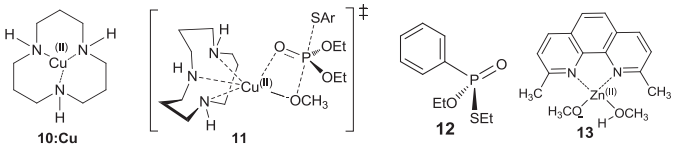
Until recently it was not experimentally known whether the attack of hydroxide and alkoxide nucleophiles on phosphonothioates such as **12** with poor leaving groups was stepwise or concerted, but a recent report by Kuo and Glazier [40] showed that the methanolytic cleavage of optically active *O*-ethyl *S*-ethyl phenylphosphonothioate **12** proceeded with inversion of configuration. This is consistent, but not uniquely-so, with the approaching methoxide attacking colinear with the departing *S*-ethyl group in an S_N2 concerted or stepwise fashion. It is notable that the methanolysis reactions catalyzed by La(III) or the 2,9-dimethylphenanthroline:Zn(II):($^-OCH_3$) complex **13** also yield completely inverted product, suggesting these also proceed by concerted S_N2 processes, or via attack of the metal delivered methoxide anti to the S-Et leaving group in a short-lived complex-stabilized 5-coordinate intermediate that does not undergo pseudorotation prior to expulsion of the leaving group.

4.2. Mechanistic studies of phosphorothioates with S in a non-bridging position

4.2.1. Metal ions and neutral phosphorothioate triesters 1a,X = S, R_1 = R_2 = CH_3, Et

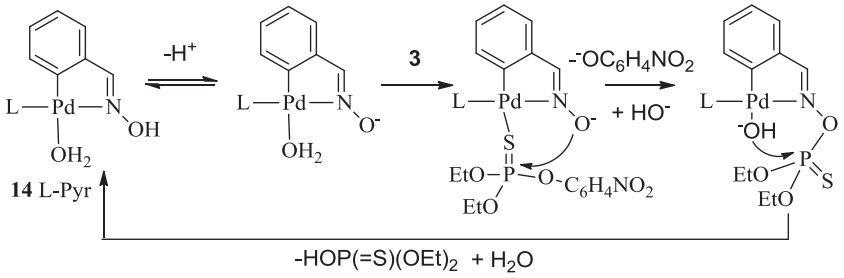
Neutral phosphorothioate triesters have received considerable attention due to their importance as insecticides and acaricides, and the desire to create effective methods for remediation of contaminated sites and materials. These materials, and their decompositions mediated by metal ions, are also of interest biologically since the dinuclear Zn(II) phosphodiesterase enzyme found in the soil-dwelling bacterium *Pseudomonas diminuta* has been shown to degrade pesticides such as paraoxon (**1a**,X = O,R_1 = R_2 = Et,X = *p*-nitro) and parathion **3**, as well as chemical weapons and substrates **7** having a bridging S [41,42]. Early studies investigated metal ion mediated hydrolysis of neutral P=S containing phosphorothioate triesters promoted by Cu(II) containing polymers [43], divalent metal ions [44], and Hg(II) [45]. However, the most actively studied and potent catalytic systems are those containing soft metal ions (in the Pearson Hard–Soft sense, [46]) such as Pd(II) and Pt(II) [47,48]. Ryabov and co-workers [47] reported that palladacycle **14** (and its corresponding Pt derivative) containing an α-nucleophilic oximate reacted quickly with phosphorothioates such as parathion (**3**) via the proposed pathway given in Scheme 5, producing a transient phosphorylated oxime which subsequently decomposed with involvement of an intramolecularly delivered Pd–$^-$OH. The proposed role of **14** was two-fold: to transiently bind the phosphorothioate through 'soft–soft' interactions, and to provide the intramolecular nucleophile(s) to cleave the bound substrate.

Subsequently, Gabbaï [49] investigated the activity of other Pd-containing catalysts such as the *C,N*-palladacycle of phenyloxazoline and a *C,N*-2-(2-pyridyl)palladacycle, and showed that the catalytic activity did not require the presence of the oxime. The latter palladacycle promotes the hydrolysis of methyl parathion (*O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate), **1a**, (X = S,R_1 = R_2 = CH_3,Y = *p*-NO_2) apparently by way of an intramolecular attack of a Pd-coordinated $^-$OH nucleophile in the Pd–S-coordinated complex **15** to give the dimethoxy phosphonic acid, $(MeO)_2(PO_2^-)$ [49c]. For the catalysts studied, several forms of the palladacycle were noted, including product bound dimeric and monomeric Pd forms.



The solvolysis of P = S containing phosphorothioates catalyzed by [Pd(II)(2-(*N,N*-dimethylaminobenzylamine)-C^1,N)(pyridine)(triflate)] in methanol simplifies some of the aqueous chemistry where the palladacycles studied had limited solubility in H_2O, and in some cases experienced inhibition since the reaction products were anionic phosphorothioate diesters which can bind more tightly to the catalyst than the initially neutral substrate. Our initial report [50], and subsequent related ones, [51,52] showed that the palladacycle Pd(II)(2-(*N,N*-dimethylaminobenzylamine)-C^1,N)(pyridine)(triflate), in methanol under basic conditions immediately forms **16** which gives impressive accelerations of the methanolysis of neutral phosphorothioates such as **2** and **3** without marked product inhibition. This is because the **16**-promoted cleavage of these triesters ($(RO)_2P$ = S(OAr)), bound as in **17**, generates a neutral product ($(RO)_2P$(=S)(OMe)) that binds no more tightly to the catalyst than does the original substrate.

A subsequent linear free energy study [53] of the **16**-catalyzed methanolysis of a phosphorothioate series with different OAr groups showed that the mechanism is considerably more complicated than had been previously assumed. Fig. 4 shows the Brønsted plots of the second order rate constant (k_2^{cat}) for the catalyzed cleavage of the series of phosphorothioates **18a–g**, and that for the corresponding methoxide reaction. Two salient points are the break in the top plot signifying a process where there is at least one intermediate, the formation and breakdown of which is rate limiting for different substrates, and the steepness of the descending portion of the Brønsted plot for substrates **18e–g**, $\beta^{Lg} = -1.93$. The vertical separation of the two plots indicates the catalysis provided by **16** is very efficient, being $(1.3-47)\times10^5$ greater than the corresponding k_2^{-OMe} rate constants for the methoxide-promoted reactions. Through the application of detailed computations, the mechanism shown in Scheme 6



Scheme 5. Proposed pathway for the cleavage of parathion (**3**) mediated by palladacycle **14** (L = pyridine); redrawn from ref. [47].

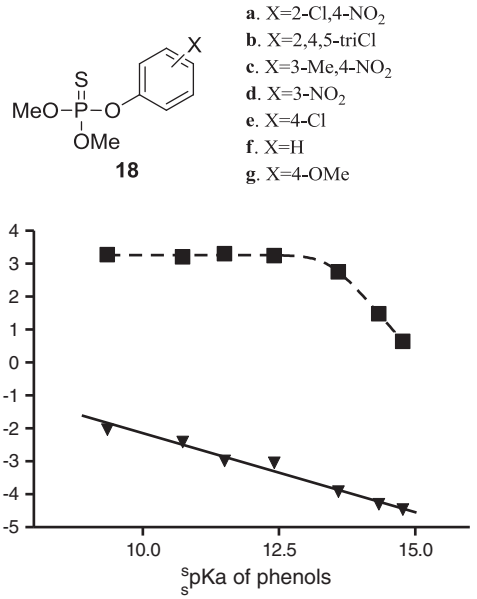


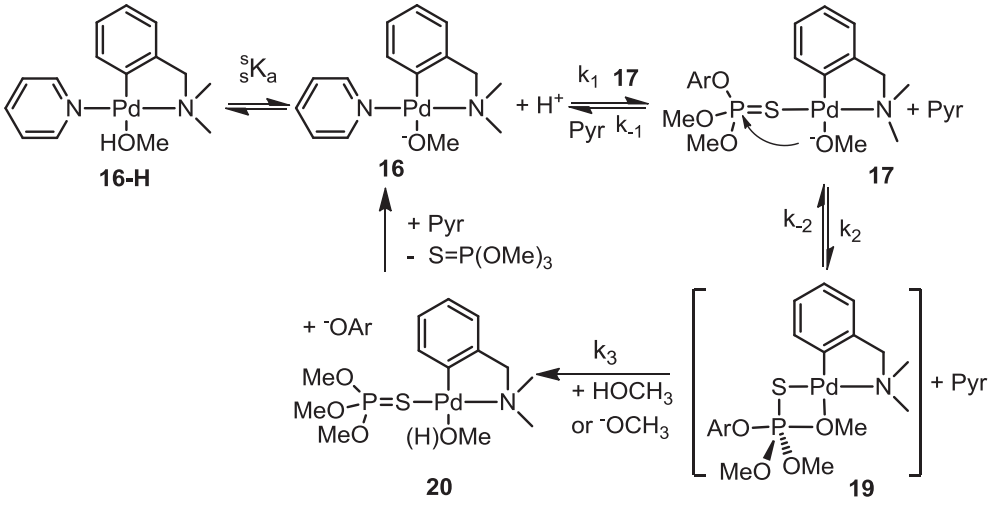
Fig. 4. A Brønsted plot of log k_2^{cat} vs. $^{s}_{s}pK_a^{HOAr}$ for the reaction of substrate **18a–g** catalyzed by palladacycle **16**, T = 25 °C. The dashed line through the data is generated from a NLLSQ fit to provide two β^{Lg} values of 0.00 ± 0.02 and −1.93 ± 0.06; $r^2 = 0.9993$. The lower linear plot (log $(k_2^{-OMe}) = (-0.47 \pm 0.03)\,^{s}_{s}pK_a^{HOAr} + (2.5 \pm 0.4)$; $r^2 = 0.9755$) is for the k_2^{-OMe} constants for the methoxide-promoted reaction of the same substrates. The $^{s}_{s}pK_a^{HOAr}$ values for **18a–g** are presented in descending order from left to right. Redrawn from data presented in ref. [55].

emerged, indicating a pathway having several intermediates (**16**, **17**, **19** and **20**), the most interesting of which (**19**), with substrates **18e–g**, was computed to be a stabilized palladacycle-coordinated 5-coordinate thiophosphorane intermediate [55].

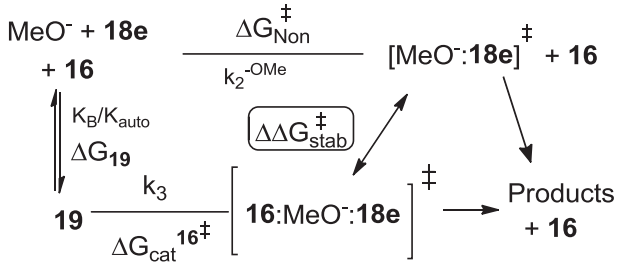
The pathway shown in Scheme 6 was subsequently supported by the results of a detailed study of the methanolysis of **18a–g** promoted by **16** as a function of $^{s}_{s}$pH [54]. That study determined that the maxima of the bell-shaped $^{s}_{s}$pH/rate profiles for the substrates moved to progressively higher $^{s}_{s}$pH on progressing from good to poorer leaving groups. The Brønsted plot of the log ($k_2^{cat\ max}$) values determined at the maximum of each substrate's $^{s}_{s}$pH/rate profile vs. the $^{s}_{s}pK_a^{HOAr}$ for the leaving groups still revealed a downward break indicative of a change in rate limiting step in a process with at least one intermediate, but with β^{Lg} values of 0.01 ± 0.01 and −0.96 ± 0.06. The latter value, corresponding to breakdown of an intermediate with expulsion of the O-aryl leaving group, was far more reasonable than the previous one of −1.93. The complicated process in Scheme 6, consistent with all the available computational and experimental data, indicated there were three classes of substrate, and three changes in rate limiting step. Nevertheless, the Brønsted plot in Fig. 4 can be satisfactorily fit by two lines, and does not require three. This is probably because there is only one example, **18a**, falling into the first class of substrates, and this may accidentally be indistinguishable from the second class of substrate in terms of its Brønsted dependence. For the fastest reacting substrate, **18a**, the rate limiting step involved replacement of pyridine from **16** (k_1) and the formation of **17**, followed by fast intramolecular attack and breakdown of intermediate **18** to product. The reaction of substrates **18b–d** involved equilibrium formation of **17** with rate limiting formation of **19** (k_2) which quickly expelled the O-aryl group. Having progressively poorer leaving groups, **18e–g** undergo equilibrium formation of the palladacycle bound thiophosphorane **19** with rate limiting expulsion of the O-aryl leaving group (k_3).

Scheme 6 suggests that there is a potentially corroborative observation, where the putative Pd-bound thiophosphorane intermediate might be observable high enough [16] to drive the $k_1/k_{-1}{}^{*}k_2/k_{-2}$ equilibria toward saturation formation of **19**. Saturation kinetics are indeed observed for the reaction of the 4-chloro derivative **18e**, where plots of the k_{obs} vs. [16] at three :pH values between 11.7 and 12.4, are downward curving. Stopped-flow measurements at high concentrations of **16** at $^{s}_{s}$pH 12.0 indicated that formation of an equilibrium mixture of the intermediate (suggested to be **19**) was rapidly formed ($k_{formation} = 87\ s^{-1}$), with a slower breakdown ($k_{obs} = 0.14\ s^{-1}$) to form the observed phenol/phenoxide product and **20**.

The rich chemistry revealed in the latter linear free energy study allows one to assess the various catalytic constants in detail and provide a quantitative assessment of how the catalytic prowess is achieved in thermodynamic terms of free energies [55]. We consider the process in Scheme 7 with substrate **18e** and the solvated palladacycle which does not contain pyridine (simply designated as **16**:$(HOCH_3)_2$ without the pyridine and methoxide of **16** in Scheme 6). The $\Delta G^{\ddagger}_{non}$ value of the methoxide reaction with **18e** comes from its k_2^{-OMe} rate constant of $(1.22 \pm 0.03) \times 10^{-4}\ M^{-1}\ s^{-1}$. The binding of both methoxide and **18e** to **16**: $(HOCH_3)_2$ is given as $\Delta G_{\mathbf{19}}$ is the free energy for formation of **19** from $^{-}$OMe, **16**:$(HOCH_3)_2$ and **18e** and is numerically equal to K_B/K_{auto} where $K_B = [\mathbf{19}][H^+]/([\mathbf{16}{:}(HOCH_3)_2][\mathbf{18e}]) = [\mathbf{19}]K_{auto}/([\mathbf{16}{:}(HOCH_3)_2][\mathbf{18e}][^{-}OMe])$. K_{auto} is the autoprotolysis constant of MeOH ($10^{-16.77}\ M^2$). $\Delta G^{\mathbf{16}\ddagger}_{cat}$ is the free energy of activation of the maximum k_3 rate constant ($0.22\ s^{-1}$) (Schemes 6,7) for the cleavage of **19**. Finally, $\Delta\Delta G^{\ddagger}_{stab}$ represents the free energy of binding of the transition state



Scheme 6. Generalized pathway for the **16**-mediated cleavage of substrates **18** as a function of the poorness of the poorness of the leaving group as judged by the $^{s}_{s}pK_a^{HOAr}$ of the conjugate acid of the leaving phenoxide.



Scheme 7. A thermodynamic cycle for comparing the palladacycle **16**:$(HOCH_3)_2$-promoted reaction and the methoxide-promoted reaction for substrate **18e**. For simplification of the diagram, **16**:$(HOCH_3)_2$ is simply designated as **16**.

comprising $MeO^- + \mathbf{18e}$ by the palladacycle. Eq. (2) shows the relationship for computing the $\Delta\Delta G^{\ddagger}_{stab}$.

$$\Delta\Delta G^{\neq}_{stab} = \left(\Delta G_{18} + \Delta G^{16\neq}_{cat}\right) - \Delta G^{\neq}_{Non} = -\left[\frac{(k_3)(K_B/K_{auto})}{k_2^{-OMe}}\right] \quad (2)$$

Fig. 5 displays a pictorial representation of the free energies for the various processes in Scheme 7. Intermediate **19** lies −12.1 kcal/mol lower in energy than free palladacycle, methoxide and substrate **18e**. Some of this energy lowering comes from the strong binding of the methoxide to the palladacycle as **16**:($^-OCH_3$):(**18e**) and more from cyclization within the complex to form **19**. Additional numerical values for the diagram come from the computed activation energies associated with k_3 (with substrate **18e**) of 18.3 kcal/mol, and k_2^{-OMe} of 22.8 kcal/mol. The $k_3 = 0.22\ s^{-1}$ corresponds to an activation energy ($\Delta G^{16\ddagger}_{cat}$) for P–OAr bond cleavage from **19** of 18.3 kcal/mol, placing the TS for breakdown of **19** 6.2 kcal/mol higher than the free energies of the three reactants at standard state. The $\Delta\Delta G^{\ddagger}_{stab}$ of 16.2 kcal/mol is the difference in energy between the 6.6 kcal/mol and the 22.8 kcal/mol activation energy for the methoxide reaction ($\Delta G^{\ddagger}_{Non}$), and represents one measure of the energy of association of the palladacycle with the $[MeO^- + \mathbf{18e}]^{\ddagger}$. Moreover, considering that the $[MeO^- + \mathbf{18e}]^{\ddagger}$ is phosphorane-like, its stabilization by binding to the palladacycle to form **19** is 34.9 kcal/mol. This is an unusual case where it appears that binding of a transition state, or a possible high energy 5-coordinate phosphorane intermediate, to a catalyst converts the latter into an observable intermediate. Importantly, the catalyst appears to be binding the 5-coordinate anionic thiophosphorane sufficiently tightly that the rate limiting step now becomes expulsion of the leaving group.

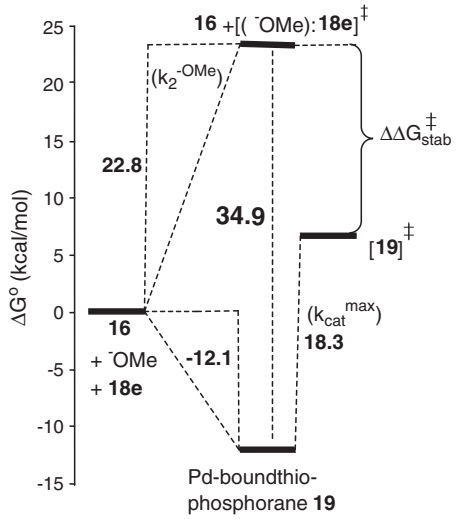


Fig. 5. An activation energy diagram for the palladacycle **16**:$(HOCH_3)_2$-catalyzed methanolyses of phosphorothioate **18e** at a standard state of 1 M and 298 K. For simplification, **15**:$(HOCH_3)_2$ is represented in the diagram simply as **16**.

4.2.2. Metal ions and anionic phosphorothioate diesters **1b**, *X = S, $R_1 = CH_3$, $R_2 = {}^{(-)}$*

There are a few reports describing metal catalyzed solvolytic reactions of anionic phosphorothioate diesters **1b**,X = S,$R_1 = CH_3$,$R_2 = {}^{(-)}$ containing sulfur in a non-bridging position. The transesterification of uridyl (3′,5′)uridine containing a phosphorothioate diester linkage is promoted by Zn(II), Cd(II), and Gd(III) in water, exhibiting rate accelerations for cyclization of up to 3600 relative to the background reaction at pH 5.6 and 363 K [56]. The metal ions Cd(II) and Mn(II) promote the methanolysis of *O*-methyl *O*-4-nitrophenyl phosphorothioate **1b**,X = S,$R_1 = CH_3$, $R_2 = {}^{(-)}$ with rate accelerations of ~10^6 relative to the background methoxide promoted reaction [57]. Even more efficient catalysis is achieved with the palladium complex **16**:($^-OCH_3$)($HOCH_3$) which promotes cleavage of *O*-methyl *O*-4-nitrophenyl phosphorothioate with a rate acceleration of ~10^{11} over the base promoted reaction [57]. A Brønsted plot for the **16**:($^-OCH_3$)($HOCH_3$) catalyzed cleavage of anionic phosphorothioate diesters led to a β^{Lg} of −0.86 indicating a significant extent of P–OAr bond cleavage in the rate limiting transition state. A more complete analysis of the linear free energy data is complicated by the absence of a known β^{Eq} for the reactions under question. Interestingly, methanolysis of *O*-methyl *O*-aryl phosphorothioate diesters promoted by the palladium complex **16**:$(CH_3OH)_2$ at ${}^{s}_{s}$pH 7.7 leads to a Brønsted coefficient of β^{Lg} of −0.01. The near zero value of β^{Lg} is consistent with (but not exclusively so) a mechanism involving rate limiting ligand exchange or intramolecular rearrangement prior to chemical cleavage of the P–OAr bond in the low ${}^{s}_{s}$pH region [56].

5. Conclusions

This brief account deals with the current state of thinking about the solvent mediated reactions of phosphorothioates, and some of the metal ion catalyzed reactions of these. The understanding of the mechanisms of the metal ion catalyzed reactions is still far from complete, particularly with the diesters and monoesters. Not surprisingly, the most effective metals for cleavage of phosphorothioates where the S is in a non-bridging position are in the soft category, such as Cu(II), Hg(II), Cd(II), Pd(II) and Pt(II). However, when the S is in a bridging position, even hard metal ions like La(III) are very effective catalysts for the methanolysis of triesters and diesters. The hydrolytic processes promoted by any of these metal ions are difficult to study under turnover conditions of excess substrate. This is due to possible inhibition from formation of a HO-containing acid product which dissociates to an anionic form having one more negative charge than the starting substrate, and thus can effectively bind to the catalyst. However, alcoholysis reactions are easier to study since this sort of problem with product inhibition is avoided. This has allowed some detailed studies of the cleavage of phosphorothioate P=S and P–SAr containing triesters where a impressive catalysis exhibited by palladacycles and La(III) species. Conspicuously under-represented in the small molecule studies are examples where metal ions are demonstrated to promoted hydrolysis or alcoholysis of phosphorothioate mono-esters. This is also observed in phosphate monoester chemistry, and is attributed to the fact that there will be strong electrostatic interactions between the dianionic substrate and electropositive metal ion that drain negative charge from the LgOP(=O)O^-S^- group which is required to assist in ejecting the OLg. Future studies attempting to address this problem will need to concentrate on catalytic systems where the metal ion exerts its influence by assisting the departure of the leaving group.

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